Azinomycin A and B Epoxyamide Stereochemistry: Relevance to DNA Sequence Selectivity

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by

Robert L. Woodward Jr.

The Ohio State University
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Project Advisor: Professor Robert S. Coleman, Department of Chemistry

ABSTRACT

Natural products have become increasingly important as anticancer drugs since the clinical introduction of mustards and actinomycin D in the mid-20th century. In fact, over 60% of the chemotherapeutics currently used are natural products or derived from them. Many of these agents exert their biological effects through covalent modification of DNA. Several studies have revealed that the manner in which this modification takes place depends largely on the stereochemistry of the given compound. As such, we sought to understand the role of stereochemistry in the interaction of DNA with the azinomycins, a class of potentially useful antitumor agents.

The azinomycins represent a small class of compounds which form interstrand cross-links within the major groove of DNA. Cross-linking occurs through reaction of two electrophilic functional groups—an epoxide and aziridine moiety—with nucleophilic N7 regions of guanines. Despite the necessity of the aziridine for cross-linking, however, it is actually the epoxide residue which primarily contributes to the cytotoxic nature of these agents. Therefore, a series of truncated structures were prepared which lacked the aziridine residue, yet maintained the biologically important epoxide moiety. Not only did these molecules provide a simpler synthetic pathway, but they also introduced varying stereochemistry, enabling us to consider stereochemical effects on DNA binding.

To provide an initial indication of how the agents approach DNA during binding, a competitive binding assay was employed. This assay utilized a DNA duplex which contained two triplet repeats of guanines and cytosines which were oriented in opposite directions. The truncated natural product was observed to preferentially bind to the most nucleophilic guanine. Its enantiomer, however, selected for a guanine in the other triplet repeat for which the truncated natural product had shown little affinity. Similarly, the enantiomer showed little affinity towards the highly nucleophilic guanine selected by the truncated natural product. This suggested that each agent was approaching the duplex in a unique manner, giving rise to the different observed selectivities. In order to confirm this result, molecular modeling studies have commenced. Initial results support the selectivity of each of the compounds but do not reveal well-defined directional orientations (5′→3′ or 3′→5′) for the agents. However, these studies do demonstrate the importance of stereochemistry in defining the interaction of an agent with DNA.

Dedicated to my parents, Bob and Jane Woodward, for their support and prayers, and to my fiancée, Megan Landerholm, whose mere presence has strengthened me throughout difficult times.

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I would first like to give all thanks to my Lord and Savior Jesus Christ. You are "my rock and my fortress and my deliverer; my God, my strength, in whom I will trust; my shield and the horn of my salvation, my stronghold." Thank you Lord for blessing me to have known and experienced you over the last few years. Secondly, my family deserves much gratitude. Without the support and encouragement of each of them, it would have been extremely difficult to make it through these four years. I would also like to thank my wife-to-be, Megan Landerholm. The joy you have filled my life with over the past two years is indescribable. Finally, I would like to thank the Coleman research group, specifically Dr. Robert S. Coleman, for allowing me to have the opportunity to find my passion in the form of bioorganic chemistry.

VITA

August 21, 1983…… Born — Gallipolis, Ohio

2006……..……. B.A. Chemistry, B.S. Biology,
The Ohio State University

PUBLICATIONS

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FIELDS OF STUDY

Major Field: Chemistry

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LIST OF ABBREVIATIONS

ILS- increase in lifespan

ISC – interstrand cross-link

PAGE- polyacrylamide gel electrophoresis

CHAPTER 1

OVERVIEW OF THE AZINOMYCINS

1.1. Introduction

During the 1960's, the National Cancer Institute began a program involving the biological screening of extracts taken from various natural sources. Not only did this study lead to the discovery of drugs such as taxol, an effective chemotherapeutic agent used in the treatment of breast and ovarian cancer, but it also opened the door for further research in the field of natural product chemotherapy.[1] One example of such research can be seen in the investigation of a unique class of natural product antibiotics, the azinomycins **(**Figure 1.1**)**.

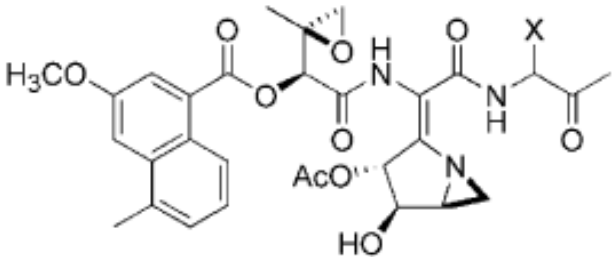

Figure 1.1: Structures of azinomycin A (X = H) and azinomycin B (X = CHO).

Azinomycins A and B were first isolated from culture broths of *Streptomyces griseofuscus* in 1986.[2,3] Initial descriptions of their biological activity indicated potent *in vitro* cytotoxic activity and substantial *in vivo* antitumor activity. Problems arising due to a lack of stability of the unusual aziridino[1,2-*a*]pyrrolidine ring system and availability of the compounds, however, resulted in the absence of additional data.[2-4] Nevertheless, research over the past few decades has led to the total synthesis of azinomycin A and other structural analogues, allowing for further characterization of these chemotherapeutic agents.

1.2. DNA Binding

The azinomycins belong to a small class of compounds that interact with duplex DNA within the major groove.[5] Specifically, these agents form interstrand cross-links (ISC) between complimentary strands of DNA without prior activation or concomitant loss of purine or pyrimidine bases.[6] Armstrong demonstrated that such ISCs form between purine bases which are two bases removed from each other on complimentary DNA strands. The initial monoalkylation event takes place when the N-7 position of a 5′-dG binds to the azinomycin framework. Subsequent binding of the N-7 of the 5′-disposed dG or dA on the complimentary strand completes the ISC.[5] Saito and coworkers furthered this model, proposing an orientation of the agent within duplex DNA in which the aziridine residue undergoes ring-opening at C10 in the monoalkylation event. Epoxide opening at C21 via the purine base two bases removed from the former then follows. Coleman later confirmed this orientation while also demonstrating that the most effective triplet recognition sequence is 5′-d(GCC)-3′/3′-(CGG)-5′, the cross-linking

of which is shown in Figure 1.2.[7] Due to the highly nucleophilic nature of these guanine residues, sequence selectivity thus appears to be generally related to nucleobase nucleophilicity.

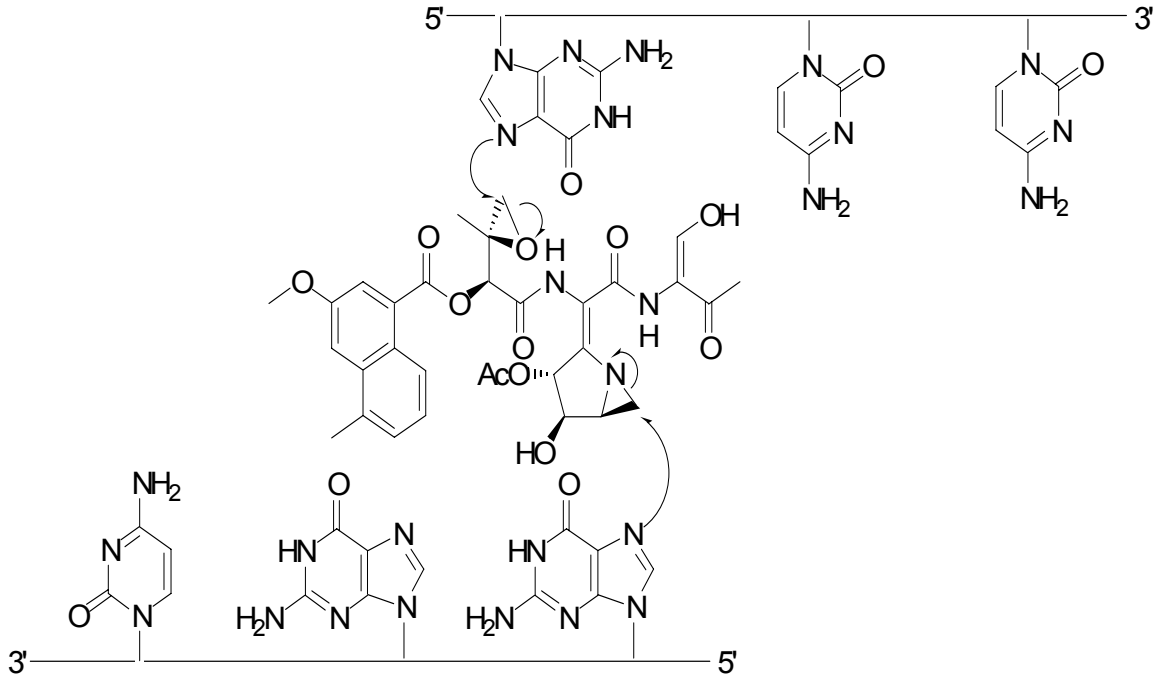

Figure 1.2: Generation of an ISC within a 5′-d(GCC)-3′/3′-(CGG)-5′ triplet pair.

1.3. Biological Activity

Initial studies of the biological activity of the azinomycins revealed that these agents possess *in vitro* cytotoxicity against the L5178 lymphoma cell line. Specifically, azinomycin B showed an IC_{50} of 0.11 μg/ml, whereas a value of 0.07 μg/ml was observed for azinomycin A. Both Gram-positive and negative bacteria were also found to be susceptible to these agents, in contrast to fungi and yeast, which were resistant. In

addition to *in vitro* activity, the azinomycins demonstrated substantial *in vivo* antitumor activity. Most notably, azinomycin B produced a 193% increase in lifespan (ILS) with 57% survivors at a dose of 16 μg/kg/day in mice with P388 leukemia. Generation of similar effects with Mitomycin C required a much larger dose (1 mg/kg/day). Substantial effects also occurred with Ehrlich carcinoma (161% ILS at 32 μg/kg/day with 63% survivors), but failed to appear with solid tumors such as Meth A fibrosarcoma.[4]

1.4. Key Structural Features

The DNA binding properties and biological activity described above rely on several distinct regions of the azinomycin framework to maintain functionality. As already mentioned, ISC formation requires the presence of the epoxide and aziridine residues to generate successful cross-linking between complimentary strands. Cross-linking, however, does not appear to be an essential feature for the azinomycins' biological effects. Hartley and colleagues instead showed that absence of the aziridino[1,2-*a*]pyrrolidine ring system did not significantly affect the IC_{50} values. Replacement of the epoxide with a cyclopropane group contrastingly resulted in a 100-fold increase in IC_{50} values in most cases. This suggests that not only is the epoxide primarily responsible for cytotoxic activity, but also that in the cell, mere monoalkylation via the epoxide residue may be sufficient to bring about the biological effects of these agents.[8]

The naphthoate moiety also possesses significant functional importance in at least two and possibly three ways. First, the substitution pattern on the naphthoate is highly relevant to the efficiency of ISC formation. Specifically, the presence of the 5′-methly group is essential in enhancing hydrophobic interactions which afford the agent its proper

orientation in the DNA duplex. Such significant interactions do not appear to exist between the duplex and the 3′-methoxy group, as only minor permutations in ISC efficiency occur with structures devoid of this group.[9] Regardless of their presence, however, sequence selectivity is in no way altered.[10]

A second possible, although highly debated, influence of the naphthoate residue results from its intercalation into the DNA duplex. This interaction was first reported to occur by Zang and Gates, who posed that the naphthoate residue weakly intercalates into the duplex.[11] Use of an incredibly low dynamic range in their measurements, as well as the absence of both positive and negative controls, led Coleman to reconsider their conclusion. His combination of viscosity, florescence energy contact transfer[12], and DNA unwinding experiments appeared to clearly indicate that intercalation in fact did not occur.[7] An additional series of unwinding experiments performed by Casley-Hayford, however, once again demonstrated the presence of intercalation.[13] Thus, it remains unclear if intercalation does in fact occur and if so, what role it plays in ISC formation.

While the role of the naphthoate is highly contested, Coleman has demonstrated a third influence from the napthoate in that its mere presence is essential for interactions with DNA. Specifically, removal of the naphthoate moiety resulted in only minute quantities of alkylation with no observed cytotoxic activity. The question of whether these effects result from the absence of intercalation or merely noncovalent interactions still lingers, however. [14]

Throughout these same studies, Coleman also discerned another important structural feature, the N16 amide nitrogen. Replacement of this species with a benzyl ester resulted in elimination of alkylation and a significant increase in IC_{50} values.

Further examination with partial structures containing two methyl groups at the N16 nitrogen revealed that alkylation yields were significantly improved, but not restored to those of species containing the amide hydrogen. It was thus concluded that while the N16 amide nitrogen is critical for alkylation, the hydrogen bonding resulting from the presence of the amide hydrogen is merely important for increasing alkylation yields.[14]

A variety of other structural features are currently under investigation to determine their contributions to the azinomycins' high degree of functionality. These include the free hydroxyl group at C-12, which is known to make azinomycin B more reactive, and the hydrogen bond between the aziridine nitrogen and the N-5 amide hydrogen which may assist in the rupturing of the aziridine residue.[10] Existence of such features would thus further the complex, integrated functions which appear in the azinomycins' compact structures.

1.5. References

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CHAPTER 2

RELEVANCE OF AZINOMYCIN "TOP-HALF" PARTIAL STRUCTURE STEREOCHEMISTRY TO SEQUENCE SELECTIVITY

2.1. Introduction

The use of synthetic analogues based upon the framework of naturally occurring antitumor agents presents a viable strategy for the development of stable and potentially potent chemotherapeutic agents. For example, the Coleman group has synthesized a series of stereoisomeric analogues which are truncated so as to remove the characteristically unstable aziridine residue (Figure 2.1). This allows the compounds to possess heightened chemical stability while still demonstrating the cytotoxic properties associated with the epoxide moiety, albeit to a slightly lesser degree (Table 2.1).[1]

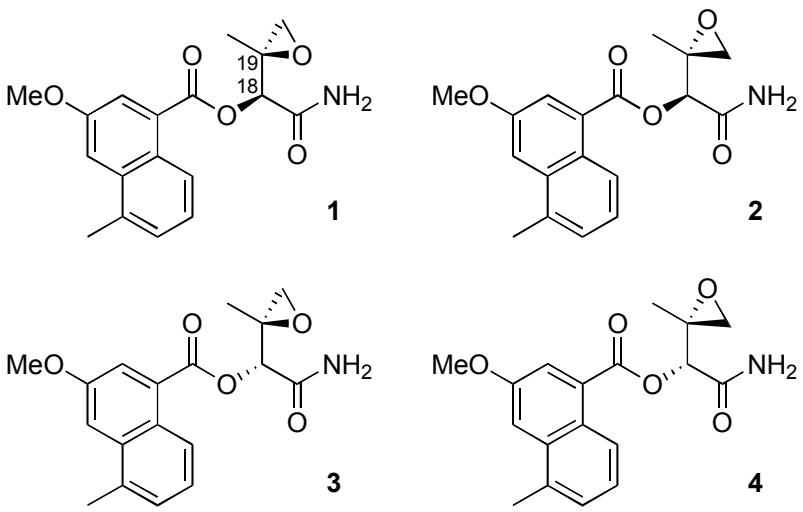

Figure 2.1: Stereoisomeric azinomycin partial structures used to study the effects of C18 and C19 stereochemistry on sequence selectivity.

Compound	MCF IC_{50} (µM)	MDA-MB-321 IC_{50} (µM)
1	1.9 ± 0.6	0.8 ± 0.2
2	2.8 ± 0.2	1.1 ± 0.3
3	2.6 ± 1.0	0.6 ± 0.1
4	2.1 ± 0.7	0.16 ± 0.07

Table 2.1: IC_{50} values for the Stereoisomeric azinomycin partial structures on MCF-7 and MDA-MB-231 cells. (Adapted from Dissertation of Edgar S. Díaz-Cruz, Ph.D., 2005)

Despite such persistence of cytotoxicity, two structural features may result in deviations of the pattern of sequence selectivity originally observed with the natural products. First, compounds **1-4** lack the aziridine residue that is involved in the initial monoalkylation event.[2] The results of this structural modification, however, have already been characterized by Coleman. He found that **1** alkylated all of the guanines to a significant but differential extent. While this revealed an altered sequence selectivity from that of the azinomycins (only the 5′-disposed guanines in the 5′-d(GCC)-3′/3′-(CGG)-5′ triplet pair were alkylated), the extent of alkylation still appeared based on nucleobase nucleophilicity.[3]

A second structural feature which may be important to sequence selectivity concerns the variability in C18 and C19 stereochemistry. Such alterations in stereochemistry are particularly important as they have been shown in some cases to alter sequence selectivity by changing the orientation with which the agent approaches DNA. For example, the natural product (+)-CC-1065 binds to DNA via a 3′-adenine-N3 alkylation event and demonstrates sequence selectivity within a three base pair region as follows: 5′-AA<u>A</u> = 5′-TT<u>A</u> > 5′-TA<u>A</u> > 5′-AT<u>A</u>. The dependency of selectivity on the two 5′ bases indicates that binding occurs in the 3′→5′ direction. The unnatural enantiomer of (+)-CC-1065, *ent*-(-)-CC-1065, however, exhibits sequence selectivity within a three base pair region as follows: 5′-A<u>A</u>A > 5′-T<u>A</u>A > 5′A<u>A</u>T, 5′T<u>A</u>T. *ent*-(-)-CC-1065 furthermore possesses strong preference that the bases 3′ from the alkylation site be A or T. Such selectivity implies that a 5′-adenine-N3 alkylation event has taken place, causing binding to occur in the 5′→3′ direction. Therefore, the differences in

stereochemistry between these two agents causes the orientation of binding to be altered, subsequently changing the pattern of sequence selectivity.[4,5]

Nevertheless, alterations of stereochemistry appear to be largely unimportant with other natural product/unnatural enantiomer pairs. Mitomycin C and its unnatural enantiomer, *ent*-mitomycin C, for example, both preferentially bind the same guanine in a 5′-CG context. This likely occurs because, in contrast to (+)-CC-1065/*ent*-(-)-CC-1065, Mitomycin C and its unnatural enantiomer possess the same orientation with respect to the DNA as they bind.[6] Therefore, the sequence selectivity of **1-4** is reported herein in an effort to begin to discern the effects of altering stereochemistry on the orientation with which the agents approach DNA during binding.

2.2. Experimental

2.2.1. Chemicals, Biochemicals, and Reagents

T4 polynucleotide kinase and T4 polynucleotide kinase buffer were obtained from New England Biolabs, Ipswich, MA. Urea, acrylamide, and sodium chloride were obtained from Fisher Scientific, Pittsburg, PA. N,N,N′,N′-tetramethylenediamine, ammonium persulfate, piperidine, 2-mercaptoethanol, and dimethyl sulfate were obtained from Acros Organics, Morris Plains, NJ. Ethylenediaminetetraacetic acid N,N′-methylene-bis-acrylamide, formamide, isopropanol, and Sephadex were obtained from Sigma, St. Louis, MO. Blue/orange loading dye (6x) was obtained from Promega Corporation, Madision, WI. γ-AT^{32}P was obtained from Amersham, Arlington Heights, IL. TBE buffer (10x) was obtained from Bio-Rad USA, Hercules, CA.

2.2.2. DNA sequences and Preparation

The 27-mer and 24-mer DNA sequences were purchased from Midland Certified Reagent Company (Midland, TX) and Integrated DNA Technologies, Inc. (Coralville, IA). They were maintained in ddH_2O following confirmation of their respective concentrations (0.1 mM) via UV-VIS analysis.

2.2.3. 5′ End-Labeling

To radioactively label the 5′-end of each DNA strand, a solution of the appropriate DNA strand (1 μL), T4 polynucleotide kinase (1 μL), T4 polynucleotide kinase buffer (3 μL), γ-$AT^{32}P$ (4 μL), and ddH_20 (21 μL) was incubated at 37 °C for 45 min. The solution was then passed through a spun column of Sephadex slurry (800 μL in a 1 mL syringe fitted with glass wool) to remove the enzyme, buffer, and other impurities. ddH_20 (170 μL) was then added to bring to the total volume to 200 μL.

2.2.4. DNA Alkylation Assay

To determine the sequence selectivity of the partial structures, a series of solutions of labeled DNA (5 μL of each strand), unlabeled DNA (1 μL of each strand), 50 mM NaCl (2 μL), and 10 mM Tris HCl pH 7.5 (20 μL) were prepared. The samples were dried in a Centrivap and redissolved in ddH_20 (20 μL). To properly anneal the DNA, the samples were heated at 95 °C for 3 min and allowed to slowly cool to room temperature in the aluminum heating block. The cooled samples were each added to a dried residue of a particular partial structure (1 μL of 10 mM) and incubated at 8 °C for an amount of time specific to the partial structure used (Compounds **1, 2, 4**: 5 h; **3**: 18 h). The samples were dried down and redissolved in 10% Piperidine (100 μL). To effect cleavage of the DNA backbone at the alkylated positions, the samples were incubated at 90 °C for 30

min. The samples were cooled at room temperature for 5 min and dried. The residues were redissolved in 3 μL of loading dye.

2.2.5. Maxam-Gilbert Sequencing

To determine the position of DNA strands resulting from alkylation and cleavage at each guanine, the banding pattern generated during PAGE was observed using the Maxam-Gilbert Sequencing Assay. A solution of 10 mM Tris HCl pH 7.5 (200 μL), tRNA (1 μL), labeled DNA (60 μL), and dimethly sulfate (12 μL) was prepared and incubated at room temperature (25 °C) for 5 min to effect alkylation. The reaction was quenched through addition of isopropanol (1 mL) and G-Stop solution (50 μL of 1.5 M sodium acetate, 1 M 2-mercaptoethanol, pH 7.0). This solution was incubated in a dry ice/acetone bath for 4 min. The frozen solution was centrifuged at 12,500 RPM for 15 min, after which the supernatant was removed. Piperidine (10%, 100 μL) was added before heating the solution at 90° C for 30 min. The solution was cooled at room temperature for 5 minutes and dried in a Centrivap. The residue was redissolved in 3 μL of loading dye.[7,8]

2.2.6. Polyacrylamide Gel Electrophoresis

To separate the fragments of DNA based on size, a solution of 50% polyacrylamide (12 mL), formamide (6 mL), urea (12.6 g), and 10x TBE buffer (3 mL) was prepared in a 125 Erlenmeyer flask. The solution was heated for 20 sec in a microwave to fully dissolve the urea. To prevent premature polymerization, the flask was then placed on ice as the gel apparatus was assembled. Two glass plates were cleaned with ethanol and placed on top of each other. Spacers were inserted between the plates along all edges except for the top and clamped into place. Following removal of

the flask from the ice bath, N,N,N′,N′-tetramethylenediamine (30 μL) and 10% ammonium persulfate (300 μL) were added to the solution. The solution was poured between the two plates after which the comb was inserted at the top. The gel cured for 30 minutes before the comb was removed and the gel was transferred to the electrophoresis chamber. The wells of the chamber were filled with 1x TBE buffer (750 mL) with which the gel wells were washed using a syringe. The gel was pre-run for 1 h at 20 W. The samples were added to the gel wells and the gel was run at 12 W for 2 h 35 min. The gel was removed from the chamber and the plates were separated. The gel was covered with Saran Wrap and placed on a phosphorimaging screen in the dark for 1 h.

2.2.7. Radioimaging

To visualize the results of PAGE, the screen was scanned with the STORM imaging system and the results displayed with ImageQuant (Molecular Dynamics, Inc., Sunnyvale, CA).

2.2.8. Statistical Analysis

Statistical and graphical analysis was performed through use of ImageQuant and Microsoft Excel (Microsoft Corporation, Redmond, WA).

2.3. Results

2.3.1. Selectivity of Compound **1**

Investigation of the sequence selectivity of **1-4** in the competitive binding assay utilized the DNA duplex seen below (Figure 2.2). This duplex not only contained the 5′-d(GCC)-3′/3′-(CGG)-5′ triplet pair used in previous studies, but also the identical triplet pair with reverse orientation, 5′-d(CCG)-3′/3′-(GGC)-5′. Such variability afforded

differing levels of nucleophilicity between the guanines, as well as structural differences within the duplex itself. A range of possible binding environments was thus offered to **1-4**. Furthermore, this sequence afforded readily distinguishable fragments following alkylation, strand cleavage, and polyacrylamide gel electrophoresis (PAGE).

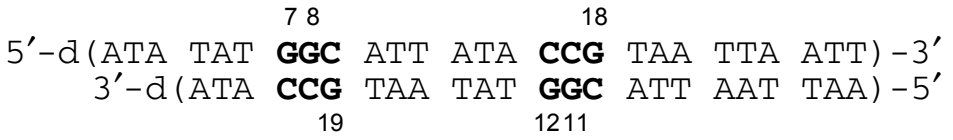

Figure 2.2: DNA sequences utilized to determine sequence selectivity.

Imaging of the gel obtained in the competitive binding assay with **1** is presented in Figure 2.3. As can be seen, the band which corresponds to alkylation at the G-7 position is noticeably darker than those of the other guanines. This indicates a higher level of radioactivity at that position and therefore a greater prevalence of that length of strand. Alkylation thus took place to a greater degree at that particular guanine. Further analysis utilizing densitometry confirmed this assumption, indicating that 35.33% of the total alkylation took place at G-7. Overall, the order of selectivity was as follows and is further illustrated in Figure 2.4: G-7 (35%) > G-11 (29%) > G-18 (14%) > G-12 (13%) > G-8 (4.4%) > G-19 (4.1%).

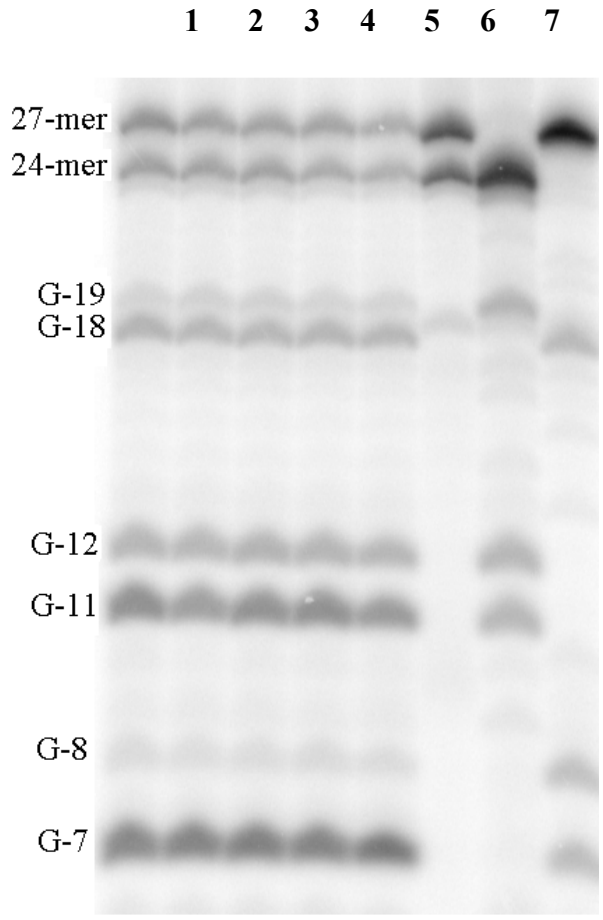

Figure 2.3: Representative gel of DNA treated with **1**. Lanes 1-5 contain DNA treated with **1** followed by piperidine cleavage. Lane 6 is the control with DNA only. Lanes 7-8 are Maxam-Gilbert G-lanes[7] which demonstrate strand lengths generated by cleavage at each guanine.

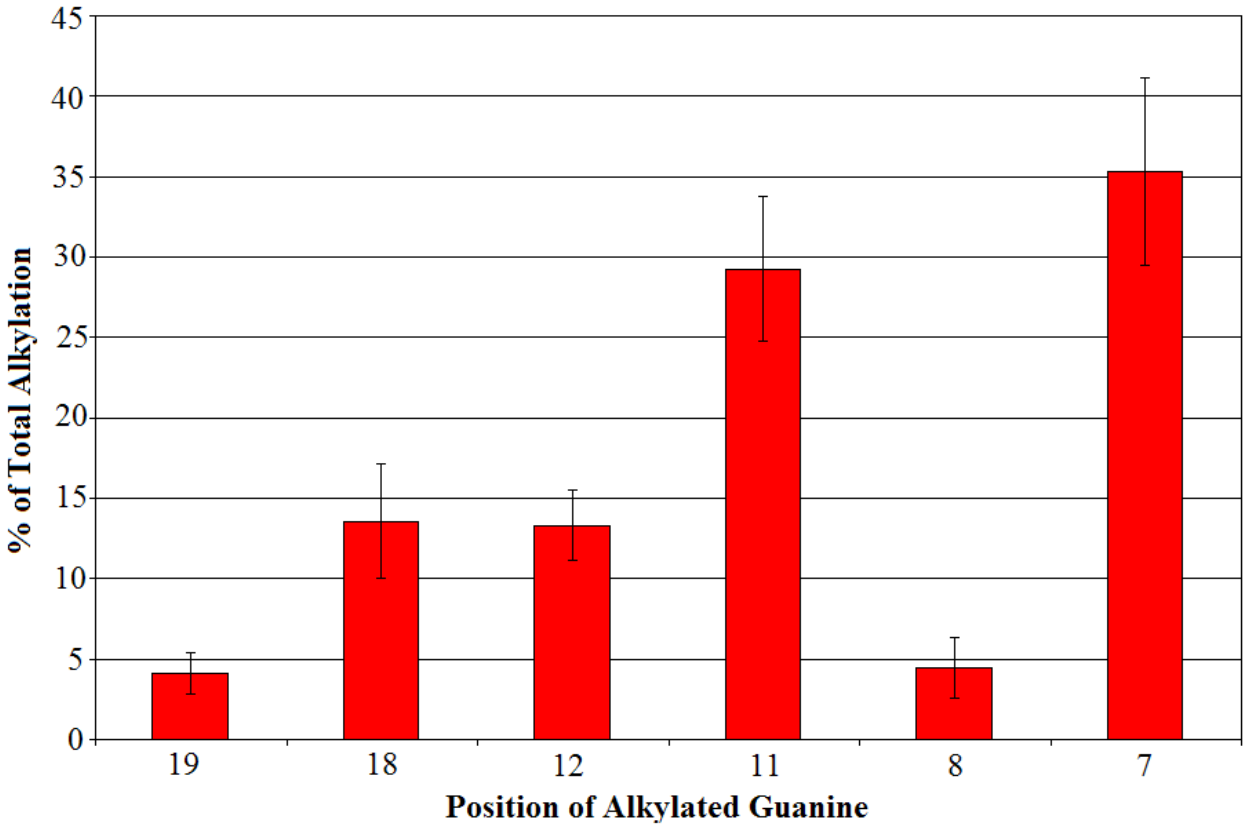

Figure 2.4: Percentage alkylation at each guanine following treatment of duplex with **1**.

2.3.2. Selectivity of Compound **2**

Figure 2.5, which shows the selectivity of **2** among the various guanines, presents a pattern of alkylation quite distinct from that observed with **1**. No single guanine appeared to be alkylated to a much greater extent than the others; rather, a moderate degree of alkylation is evident at several positions, specifically G-7, G-11, and G-18. Graphical analysis yielded an order of sequence selectivity confirming this pattern which is as follows and is presented graphically in Figure 2.6: G-11 (26%) > G-7 (22%) > G-18 (20%) > G-12 (13%) > G-19 (9.2%) > G-8 (8.6%).

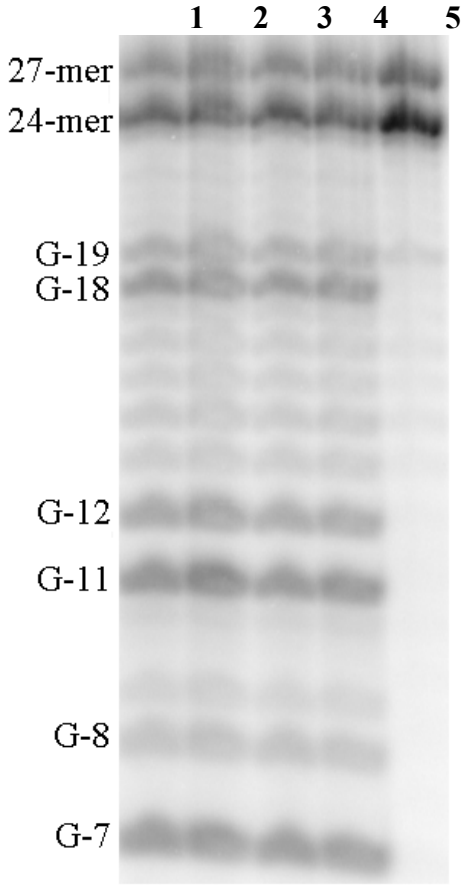

Figure 2.5: Representative gel of DNA treated with **2**. Lanes 1-4 contain DNA treated with **2** followed by piperidine cleavage. Lane 5 is the control with DNA only.

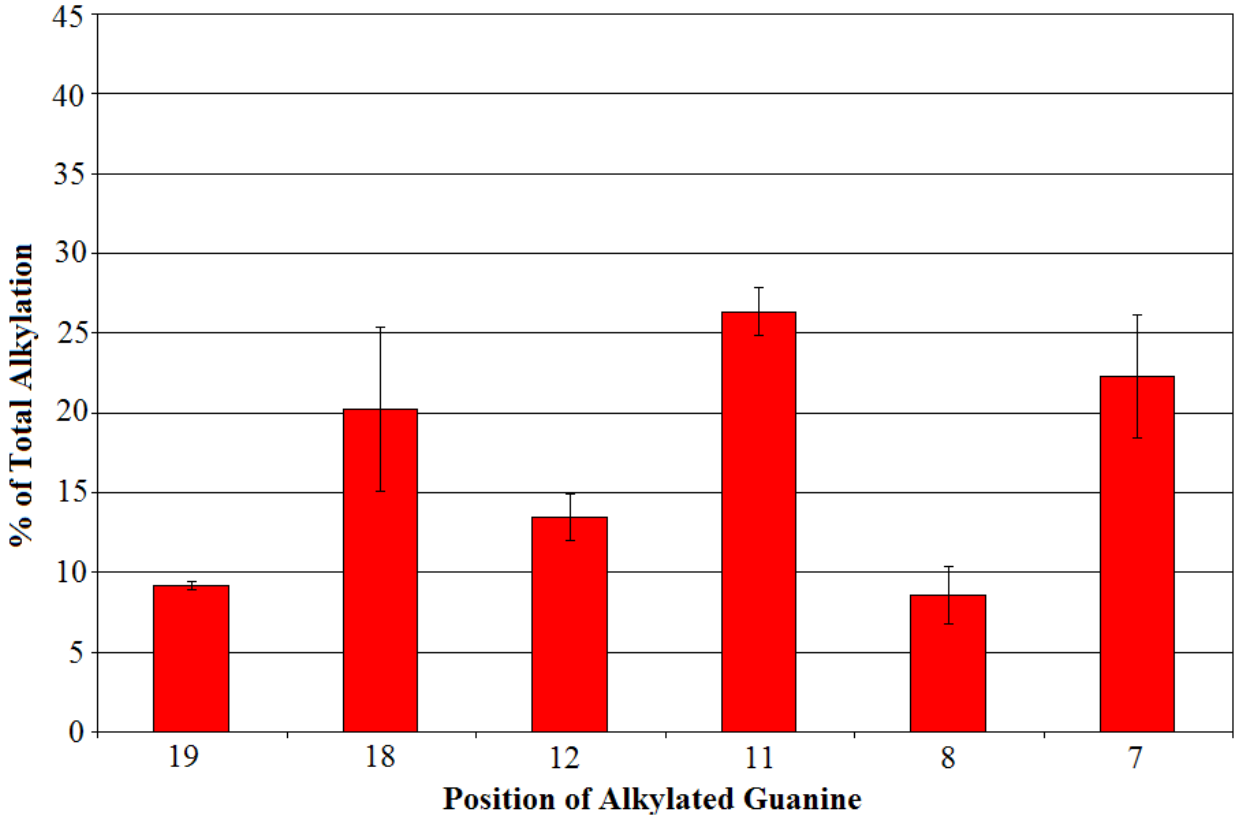

Figure 2.6: Percentage alkylation at each guanine following treatment of duplex with **2**.

2.3.3. Selectivity of Compound **3**

3 produced a banding pattern quite similar to that seen for **2** in that a high level of sequence selectivity was not noticeable for one particular guanine (Figure 2.7). Moderate levels of alkylation were instead observed, generating the following order of sequence selectivity which is also presented in Figure 2.8: G-11 (30%) > G-7 (23%) > G-18 (19%) > G-12 (14%) > G-19 (10%) > G-8 (4.8%). This order is identical to that given for **2**. However, the percent of total alkylation at the three most reactive guanines varied slightly (G-11: 26% vs. 30%; G-7: 22% vs. 23%; G-18: 20% vs. 19%).

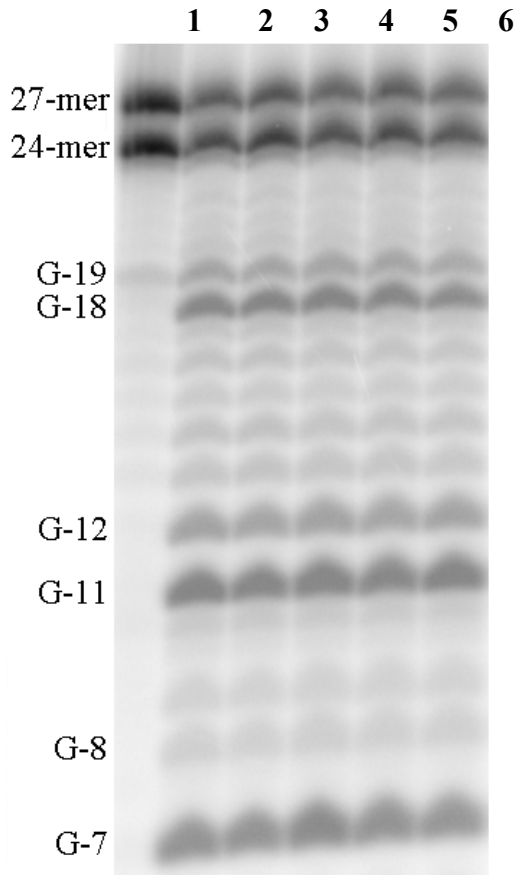

Figure 2.7: Representative gel of DNA treated with **3**. Lane 1 is the control with DNA only. Lanes 2-6 contain DNA treated with **3** followed by piperidine cleavage.

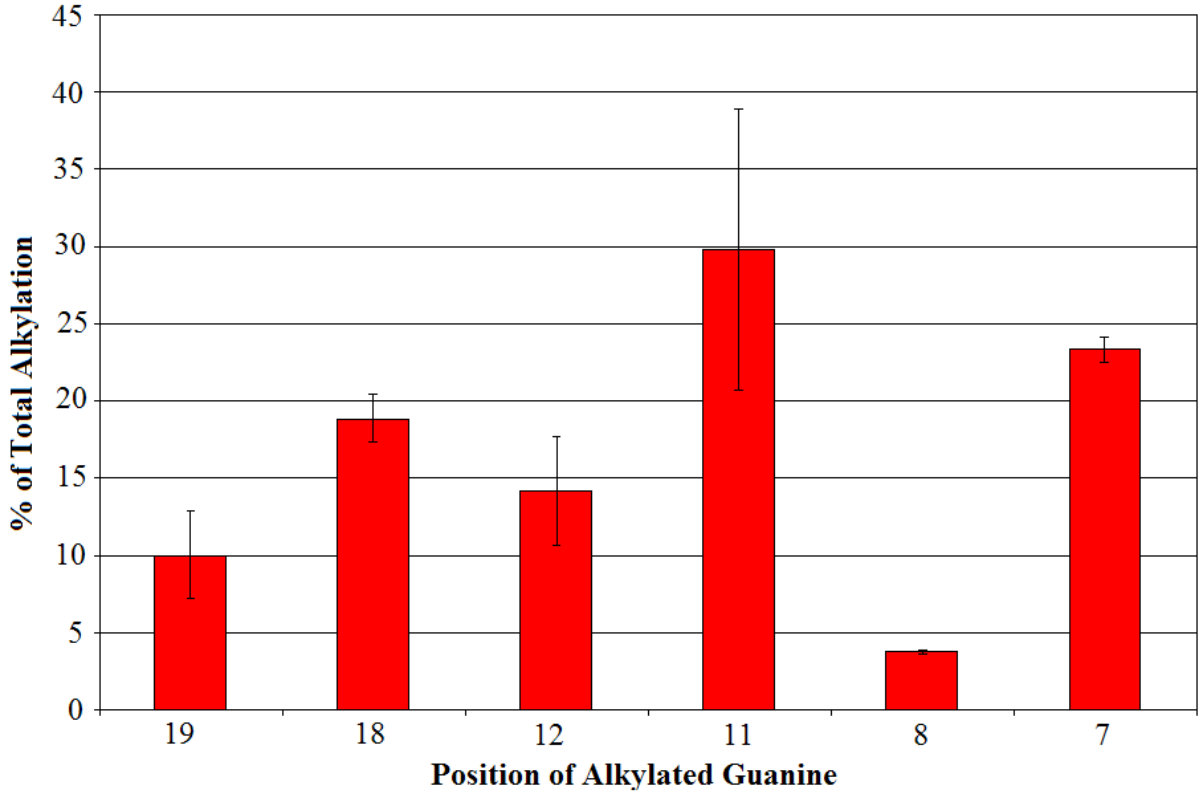

Figure 2.8: Percentage alkylation at each guanine following treatment of duplex with **3**.

2.3.4. Selectivity of Compound **4**

The banding pattern which appeared from use of **4** in the competitive binding assay revealed one noticeably darker band as was seen with **1** (Figure 2.9). In this instance, however, the darkest band corresponded to alkylation at G-18. Furthermore, the following overall pattern of sequence selectivity, which is also depicted in Figure 2.10, was quite different from that observed with **1**: G-18 (36%) > G-11 (24%) > G-12 (14%) > G-7 (11%) > G-19 (8.8%) > G-8 (6.9%). The percent of total alkylation at G-7, for example, was 35% with **1** compared to 11% for **4**. Similarly, alkylation at G-11 decreased from 29% to 24% upon going from **1** to **4**. An increase was observed, however, with G-18 as it rose from 14% to 36%.

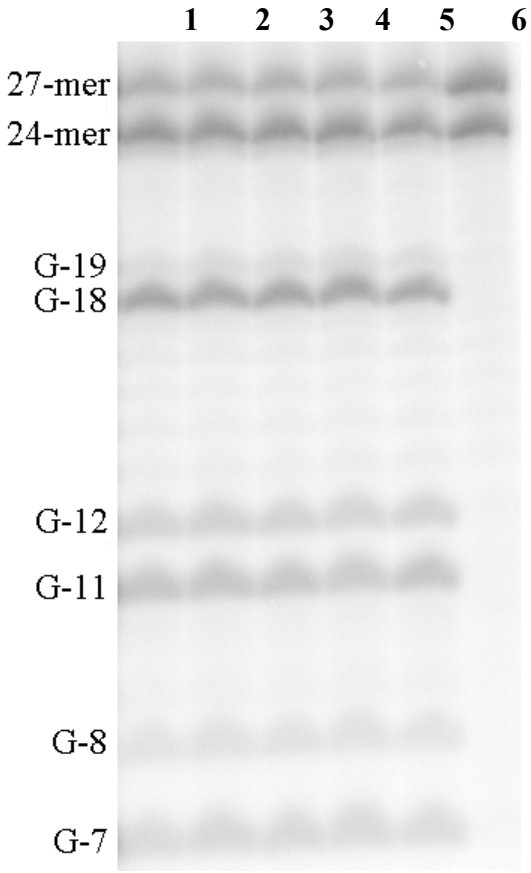

Figure 2.9: Representative gel of DNA treated with **4**. Lanes 1-5 contain DNA treated with **4** followed by piperidine cleavage. Lane 6 is the control with DNA only.

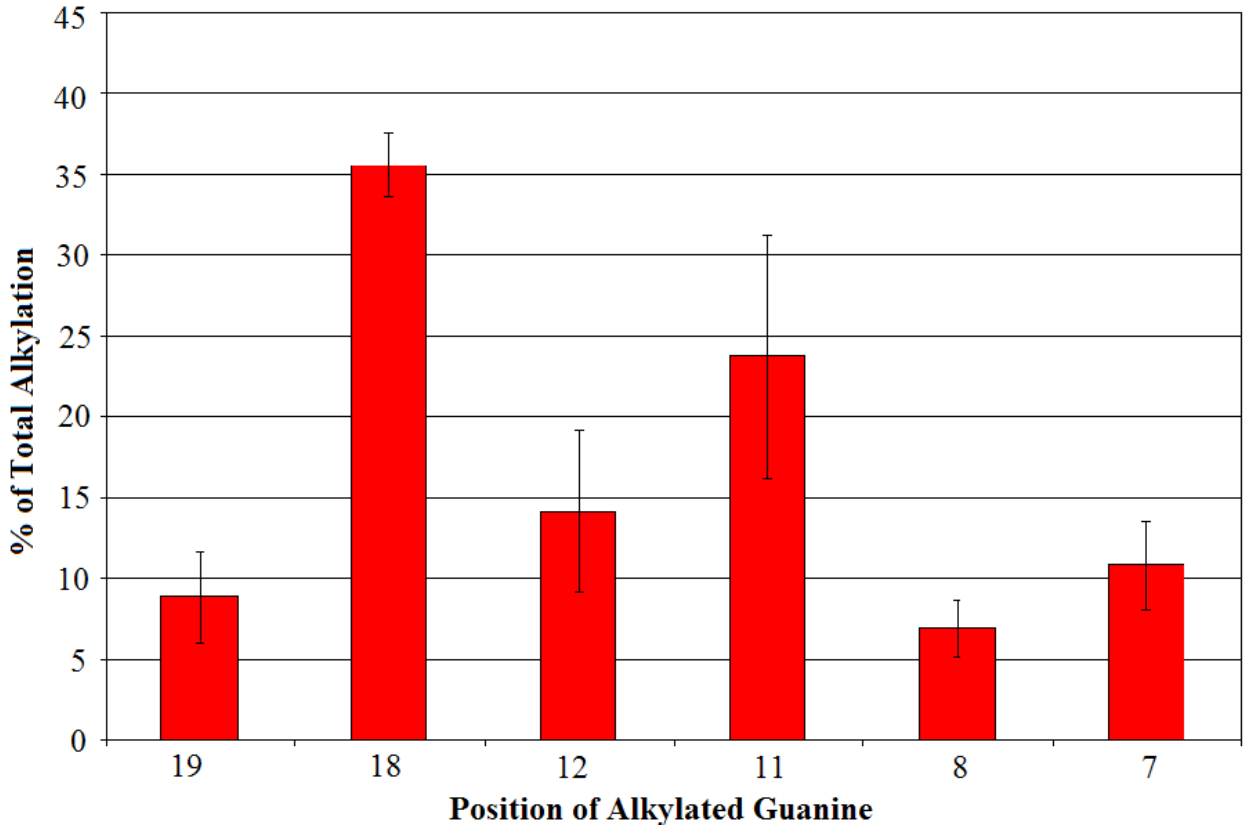

Figure 2.10: Percentage alkylation at each guanine following treatment of duplex with **4**.

2.4. Discussion

Variation in sequence selectivity among enantiomeric pairs has been shown to be related to differences in the orientation of the agents with respect to DNA during binding. Furthermore, these changes in orientation appear to largely result from the disparities in stereochemistry among the compounds, as with (+)-CC-1065 and *ent*-(-)-CC-1065.[4] Herein evidence is provided that this is in fact the phenomena being observed with the azinomycin "top-half" partial structures.

Consider first that the sequence selectivity of **1-4** varied considerably. While **1** (S,S) exhibited clear preference for G-7, **2** (S,R) and **3** (R,S) possessed a less conspicuous pattern of selectivity with G-11 receiving the highest levels of alkylation. **4** (R,R), however, acted much like **1** in that it demonstrated an obvious preference for a particular guanine, but selected for G-18 instead of G-7. This selection for G-18 over G-7 was in fact quite pronounced as G-18 received 36% of the total alkylation with G-7 receiving only 11%. The polar opposite was observed for **1** as G-7 and G-18 were alkylated 35% and 14% of the time, respectively.

Such results imply that the orientation of each agent with respect to the DNA duplex is quite distinct from that of the others. Initial modeling studies indicate that this is likely the case. This can be concluded from distinct hydrogen bonding networks which are proposed to exist between each agent and the duplex. Nevertheless, these studies do not support the idea of each orientation possessing a well-defined (5′→3′ or 3′→5′) direction with respect to the duplex. As such, it can only be concluded that the presence of distinct patterns of sequence selectivity among the partial structures results from

differing orientations with respect to the duplex which themselves result from the different stereochemistries of the agents.

2.5. Acknowledgements

This study was conducted under the supervision of Dr. Robert S. Coleman in the Department of Chemistry. Dr. Sarah B. Cortright, Dr. Pei-Hua Liu, and Dr. Mark T. Tierney synthesized the azinomycin analogues which were utilized. Amy M. Hayes and Erika A. Crane also served as collaborators, assisting in data collection and analysis.

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CHAPTER 3

CONCLUSIONS AND FUTURE WORK

Modifying the framework of naturally occurring antitumor agents presents interesting effects which may or may not be useful in the development of structural analogues. For example, elimination of the aziridine residue from the azinomycin scaffold did not act to alter the cytotoxic potential of this agent to a significant degree. Furthermore, the highly unstable nature of this moiety made this modification quite constructive. The impact of altering stereochemistry on the mechanism of action of the azinomycins was considered above. It is quite apparent that changes in stereochemistry do in fact alter the manner in which these agents interact with DNA. As mentioned previously, the enantiomer of the truncated natural product was highly selective for a guanine within the triplet pair that was oriented in the opposite direction from that associated with truncated natural product binding. The importance of this difference in binding is yet to be investigated, however. As such, several topics pertaining to this difference merit further research.

One possible direction for such research could involve examination of a possible connection between the orientation of binding/stereochemistry and cytotoxic potential. A connection such as this would then allow for development of other structural analogues which possess the stereochemistry associated with the most potent agent among **1**

through **4**. Finally, another potential avenue for research would involve synthesis of the unnatural enantiomer of azinomycin B and investigation of its binding properties. Given the results presented above, it is anticipated that it would bind with its epoxide at G18 and its aziridine at G12. If this is in fact the case, this agent will have demonstrated the quality of directional inversion upon alteration of all stereocenters, a quality which has been shown in only a limited number of cases.

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